ERDC TN-APCRP-BC-03
April 2001

Recovery of endophytic fungi from *Myriophyllum spicatum*

by Judy F. Shearer

INTRODUCTION: *Myriophyllum spicatum* L. (Eurasian watermilfoil, hereafter called milfoil) is one of many invasive plant species that are investigated by biocontrol researchers at the U.S. Army Engineer Research and Development Center, Environmental Laboratory (EL). For the past 10 years, plant samples from healthy, declining, or debilitated milfoil populations have been field-collected or have been sent to the EL specifically requesting microbial analysis, often in an attempt to explain sudden population declines. As a result of assaying the plant material for pathogens, lists of fungi associated with milfoil at each collecting site were compiled. It became apparent after examining the lists that certain fungal organisms were consistently isolated from the samples at high frequencies regardless of the condition of the host. The potential biological control pathogens were subjected to a screening regime for efficacy on milfoil. Less than 1 percent of the isolates from the initial screening have become subjects for additional testing. The fact that the majority of these fungi do not induce disease in healthy milfoil in laboratory tests but consistently appear in field populations of healthy as well as stressed milfoil suggests that they may exist as endophytes in host tissues.

The term endophyte originated with De Bary (1866) who used the term to distinguish fungi that reside within host tissues from epiphytes, fungi that live on the outer surfaces of host plants. In the 1980's, the term became restricted to include only those organisms that cause asymptomatic infections in a host and excluded pathogenic fungi and mutualists such as mycorrhizae (Carroll 1986). In recent years a broader interpretation of endophyte has led to the inclusion of organisms that have an epiphytic phase prior to internal infection, mutualistic microbes, benign commensals, and latent or quiescent pathogens that may live symptomless in their hosts for some time before manifesting themselves (Petrini 1991).

Endophytic mycobiota have been reported from a variety of hosts worldwide including trees, shrubs, grasses, mosses, ferns, and lichens (Stone, Bacon, and White 2000). They have been isolated from asymptomatic foliage, stems, bark, roots, and seeds in alpine, temperate, and tropical regions from both terrestrial and aquatic habitats. Studies of endophytic microbes over the past 25 years indicate they occupy a unique ecological niche and are thought to influence plant distribution, ecology, physiology, and biochemistry (Sridhar and Raviraja 1995). In recent years their study has intensified as researchers look to understand and improve plant fitness, to develop safe bioherbicides and to produce compounds for industrial and pharmaceutical applications (Sridhar and Raviraja (1995).

With a few exceptions, the role of endophytic fungi within plant tissues is poorly understood. In certain grasses their presence provides some insight into their ecological impact on community structure. Grass endophytes are typically systemic and perennial in the host (Clay 1997). Laboratory and field studies have shown that herbivores are deterred from feeding on infected plants resulting in changes in the richness and importance of other species in natural and agricultural grassland communities (Belesky and Malinowski 2000). When some grass and sedge species are infected by fungal endophytes in the Clavicipitaceae, they have been reported to have an increased resistance to insect pests (Clay 1988). The resistance is traceable to different tolerances to alkaloids produced

by the fungi (Lane, Christensen, and Miles 2000). Grass seeds infected by endophytes usually have higher alkaloid concentrations than photosynthetic tissues resulting in preferential feeding by both insects and birds on uninfected rather than infected seeds (Clay 1991). The endophyte-infected seeds remain extremely viable and exhibit vigorous germination (Clay 1991).

Endophytic fungi in conifers and woody perennials have been reported in some cases to deter insect feeding and antagonize pathogens (Sridhar and Raviraja 1995). Two fungal species in the genus *Lophodermium* infect needles in populations of Scots pine of mixed ages. The presence of the endophytic *L. conigenum* seems to help reduce the presence of the pathogenic *L. seditiosum* in the stands (Carroll 1991). Carroll (1991) also reported consistent evidence for antagonism between the endophytic fungus *Rhabdocline parkeri* and gall midges on needles of Douglas fir. Mortality of the midges was higher in needles infected by the fungus than in uninfected needles. In contrast, Faeth and Hammon (1997) did not find strong correlative evidence that endophytes interact mutualistically with Emory oak by increasing resistance to leafminers in the genus *Cameraria.*

Rodriguez and Redman (1997) identify four classes of endophytic fungi based on their behavior within plant tissues: 1) fungi that actively grow through host tissues, resulting in extensive colonization; 2) fungi that grow through host tissues but only colonize a small percentage of host tissues; 3) fungi that are quickly inhibited from colonization by plant defense responses and remain quiescent until the host becomes senescent; and 4) fungi that are inhibited but remain metabolically active in the host. Class three endophytes are often referred to as latent pathogens because symptoms appear in the host only under certain environmental or nutritional conditions or by the state of maturity of the host (Agrios 1988).

Most of the available information on endophytes has come from studying terrestrial plants with only a paucity of information on plants in aquatic habitats. Andrews, Hecht, and Bashirian (1982) reported a latent pathogen (*Acremonium curvulum* W. Gams) growing epiphytically as well as endophytically in milfoil in Wisconsin. The fungus was isolated from stems, leaves, and roots of healthy and debilitated milfoil. In laboratory studies, apparently healthy plants stressed by environmental or mechanical manipulations declined precipitously and concurrently with the proliferation of the fungus. As a result of field and laboratory observations, Andrews, Hecht, and Bashirian (1982) speculated that some milfoil declines could in part be attributed to the presence of the fungus in host plant tissues.

While *A. curvulum* has been notably absent from EL collections, other species of *Acremonium* have been consistently isolated in small numbers from both healthy and stressed milfoil populations. Even more common has been the presence of *Mycoleptodiscus terrestris* (Gerd.) Ostazeski. Members of both genera have been previously reported as endophytes, *Acremonium* spp. in several grasses and milfoil and *Mycoleptodiscus atromaculans* in white Atlantic cedar (Bills and Polishook 1992a). A reexamination of fungal isolate lists obtained from milfoil samples collected in the continental United States has suggested that endophytes may be extremely common in milfoil tissues. It was in large part from samples collected on the Tennessee and Cumberland River systems in 1993 and 1994 that it first became apparent that certain fungi were consistently and repetitively collected from milfoil tissues regardless of the condition of the host. The species diversity and colonization frequency of suspected endophytes in the shoot tissue of milfoil are reported here.

MATERIALS AND METHODS: Fungal associates of milfoil were tabulated from plant samples collected in 1993 on the Tennessee and Cumberland River systems where milfoil populations had remained constant since invasion, had declined, or had declined and recovered (Figure 1). Milfoil

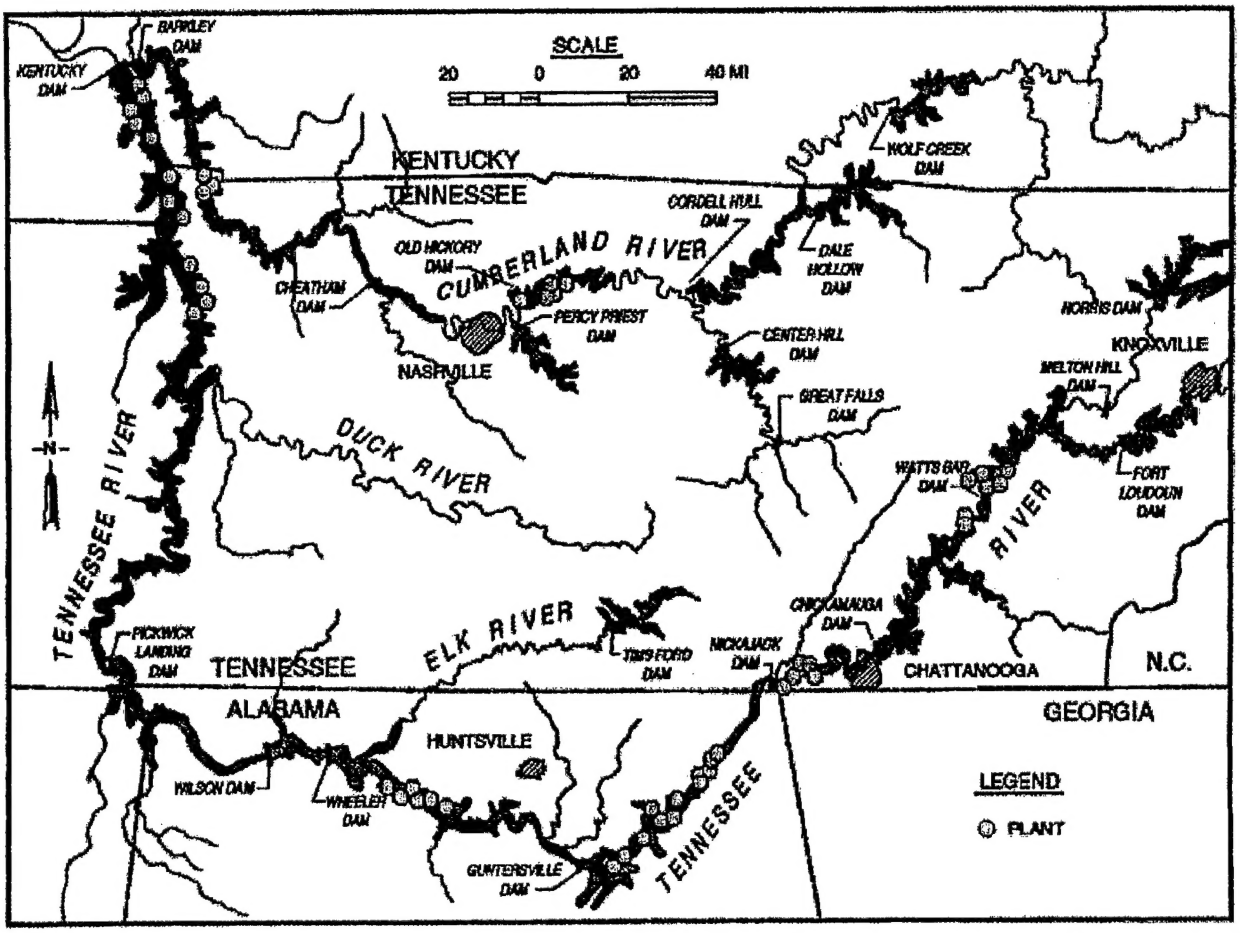

Figure 1. Sites on the Tennessee and Cumberland Rivers where milfoil plant samples were collected in 1993

infestations at the sites varied from sparse to extremely dense. At each site, an approximate 500-g sample of milfoil shoot tissue was collected, was washed to remove soil and debris, and was kept cool at approximately 5 °C until it could be bioassayed. The samples were processed in two ways: a stem bioassay and dilution plating of macerated stem and leaf tissue. For the stem assay, pieces of stem tissue approximately 2 cm long were cut from the basal, mid-section, and apical portions of five randomly selected plants. The stem pieces were washed in running water for approximately 10 min, submerged in a solution of 3.5 percent sodium hypochlorite for 3 min, and rinsed in sterile water. The three segments from each plant were placed in slits cut into a Martin's agar (H_2O, 1 L; agar, 20 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; peptone, 0.5 g; dextrose, 10 g, yeast extract, 0.5 g; rose Bengal, 0.05g; streptomycin sulfate, 0.03g) plate and incubated at room temperature for 1 week. The plates were examined daily for the development of fungal colonies on the segments. Mycelial fragments or spores from all filamentous fungal colonies that grew out of stem segments from each sample were numbered sequentially as they were transferred to Potato Dextrose Agar (PDA) (Difco Inc., Detroit, MI) slants. After 7-10 days, the slants from all sites were sorted together and

enumerated into morphological "species" based on gross colony morphology. Isolates from representative slants of a species or isolates from slants where differences in colony morphology were ambiguous were transferred to Petri dishes containing Potato Carrot Agar (PCA) (Dhingra and Sinclair 1995) or PDA for identification. Nomenclature of fungi follows that listed in Booth (1966, 1971); Carmichael et al. (1980); Domsch, Gams, and Anderson (1980); Ellis (1971, 1976); Farr et al. (1989); Gams (1971); Sutton (1980); and Pitt (1979).

For the dilution plating, a 10-g sub-sample of milfoil stem and leaf tissue from each site was thoroughly washed in running water for 10 min, submerged in a solution of 3.5 percent sodium hypochlorite for 3 min, and rinsed in sterile water. The tissue was added to 100 ml of sterile water and macerated in a blender for 30 sec. The resulting slurry was diluted to concentrations of 1/100 and 1/500. The dilutions were plated in 1-ml aliquots onto Martin's agar plates (3 plates per dilution concentration) and incubated at room temperature for 1 week. Filamentous fungal colonies growing on the plates were transferred to PDA slants and processed as described above for the stem study.

Four milfoil-inhabited sites on Guntersville Reservoir were monitored monthly between June and October 1994. The sites were selected based on visual observations and presence of relatively high levels of *M. terrestris* in milfoil tissues during the 1993 sampling trip on the Tennessee and Cumberland Rivers. In 1994, milfoil plant communities at two of the sites appeared to be in a state of decline. Throughout the collecting period, plants remained well below the water surface and exhibited sparse growth. By contrast at the other two sites, plants had grown to the water surface by June and remained vigorous and healthy in appearance until September when signs of senescence began to appear. Five approximate 100-g samples of milfoil shoot tissue were collected monthly at each site and kept cool in an ice chest until bioassayed, usually within 48 hr. The plant samples were processed using the dilution plating method described above.

RESULTS AND DISCUSSION: A total of 482 fungal isolates were cultured from milfoil stem tissue collected from 52 milfoil-inhabited sites on seven reservoirs in the Tennessee and Cumberland River systems (Table 1). *Mycoleptodiscus terrestris* was the most frequently isolated species accounting for approximately 81 percent of the total isolates, followed by *Pythium* sp. and *Acremonium* sp. at 8 percent and 3 percent, respectively. All other species were isolated at frequencies $\leq$ 1 percent. *Mycoleptodiscus terrestris* was found in all seven reservoirs. It was absent from milfoil tissues in three of six samples from Melton Hill, three of six samples from Nickajack, and one of twelve samples from Guntersville (Figure 1).

The dilution plating method yielded approximately the same number of isolates and species but differed in species composition and isolation frequency (Table 2). Again *M. terrestris* was the most frequently isolated species accounting for approximately 44 percent of the total isolates. A dematiaceous unknown, *Cylindrocarpon destructans*, *Penicillium sclerotiorum*, *Cladosporium sphaerospermum*, *Plectosphaerella cucumerina*, and *Phoma* sp. all occurred at higher frequencies in dilution plating than in the stem bioassay. Absent from the stem study, *Polyscytalum fecundissismum* made up approximately 6 percent of the total isolates from dilution plating.

Table 1
Fungi Isolated from Stem Tissue of Milfoil Collected in Eight Reservoirs Along the Tennessee and Cumberland Rivers[1]

Species	Number of isolates	Number of sites	Number of reservoirs
Mycoleptodiscus terrestris (Gerd) Ostazeski	391	47	7
Pythium sp.	39	14	5
Acremonium sp.	14	9	3
Cylindrocarpon destructans (Zins.) Scholten	6	6	4
Dematiaceous unknown	6	3	2
Glomerella cingulata (Stonem.) Spaulf. & Schrenk	3	3	3
Phomopsis archeri)S.A. Archer) Sutton	3	3	3
Emericellopsis minima Stolk	3	2	2
Curvularia pallescens Boedijn	2	2	2
Penicillium sclerotiorum van Beyma	2	2	2
Plectosphaerella cucumerina (Lindf.) W. Gams	2	2	1
Zygosporium masonii Hughes	2	1	1
Moniliaceous unknown	1	1	1
Phoma sp.	1	1	1
Cladosporium sphaerospermum Penz.	1	1	1
Cladosporium oxysporum Berk. & Curt.	1	1	1
Colletotrichum gloeosporioides	1	1	1
Microsphaeropsis olivacea (Bonord.) Höhn	1	1	1
Nigrospora sphaerica (Sacc.) Mason	1	1	1
Total	482		

[1] Species are ordered by decreasing number of isolates recovered.

Several reasons could account for the differences in species composition and frequency between the two isolation techniques. Rapid growth of one fungus from a stem piece may inhibit growth of other fungi present in the tissue. Both *M. terrestris* and *Pythium* sp. were observed growing from stem pieces within 3 days after plating. The physical grinding of plant tissues used in the dilution plating process had the potential of compromising viability of some species by disrupting fungal cell walls. The spatial distribution of the macerated tissue on the agar plates allowed slower growing species to establish individual colonies that could then be transferred to agar slants. These species might well have been overlooked or overgrown in the stem plating process. Finally, some species probably preferentially reside in leaf rather than stem tissue.

At the four sites selected for monitoring on Guntersville Reservoir in 1994, eight species were isolated from more than one site at more than one sampling interval (Figure 2). *Mycoleptodiscus terrestris* was isolated from these sites in 1993 and was consistently isolated at each collecting interval from June through October 1994 (Figure 2A). Although the isolation frequency of

Table 2
Fungi Isolated from Stem and Leaf Tissues of Milfoil Collected in Eight Reservoirs Along the Tennessee and Cumberland Rivers[1]

Species	Number of isolates	Number of sites	Number of reservoirs
Mycoleptodiscus terrestris (Gerd) Ostazeski	198	39	7
Dematiaceous unknown	58	16	5
Cylindrocarpon destructans (Zins.) Scholten	57	10	4
Polyscytalum fecundissimum Riess	27	6	2
Penicillium sclerotiorum van Beyma	20	9	5
Cladosporium sphaerospermum Penz	13	9	4
Plectosphaerella cucumerina (Lindf.) W. Gams	13	2	2
Exophiala pisciphila McGinnis & Ajello	11	1	1
Papulaspora immersa Hotson	11	1	1
Fusarium sp.	7	2	2
Microsphaeropsis olivacea (Bonord.) Hőhn	5	4	3
Phoma eupyrena Sacc.	5	3	1
Endophragmia pinicola M. B. Ellis	4	4	3
Phoma sp.	4	4	3
Acremonium tubakii W. Gams	4	4	2
Acremonium strictum W. Gams	2	2	2
Colletotrichum gloeosporioides	2	2	2
Leptosphaeria coniothyrium (Fuckel) Sacc.	2	1	1
Pseudeurotium ovalis Stolk	1	1	1
Dematiaceous unknown II	1	1	1
Emericellopsis minima Stolk	1	1	1
Pestalotiopsis guepinii (Desm.) Stey	1	1	1
Total	447		

[1] Species are ordered by decreasing number of isolates recovered.

M. terrestris was relatively low early in the season for all sites except site 1, by October it accounted for $\geq$ 50 percent of all isolates from each site. At sites 3 and 4 where milfoil plants appeared asymptomatic until senescence began, it accounted for 99 and 68 percent of the isolates, respectively, by October. In June, colonization of milfoil tissues was shared primarily with *Cylindrocarpon destructans*, *Phaeoramularia* sp., *Sclerotium hydrophilum*, *Polyscytalum fecundissimum*, and a dematiaceous unknown (Figures 2C, 2D, 2E, 2F, 2G, and 2H, respectively). With the exception of *S. hydrophilum* in sites 2 and 4 all these species became less common in milfoil tissues as the season progressed. In July, a moniliaceous unknown that was absent from all other collecting periods accounted for 3, 23, 21, and 24 percent of the isolates, respectively, for sites 1-4. Other fungi of

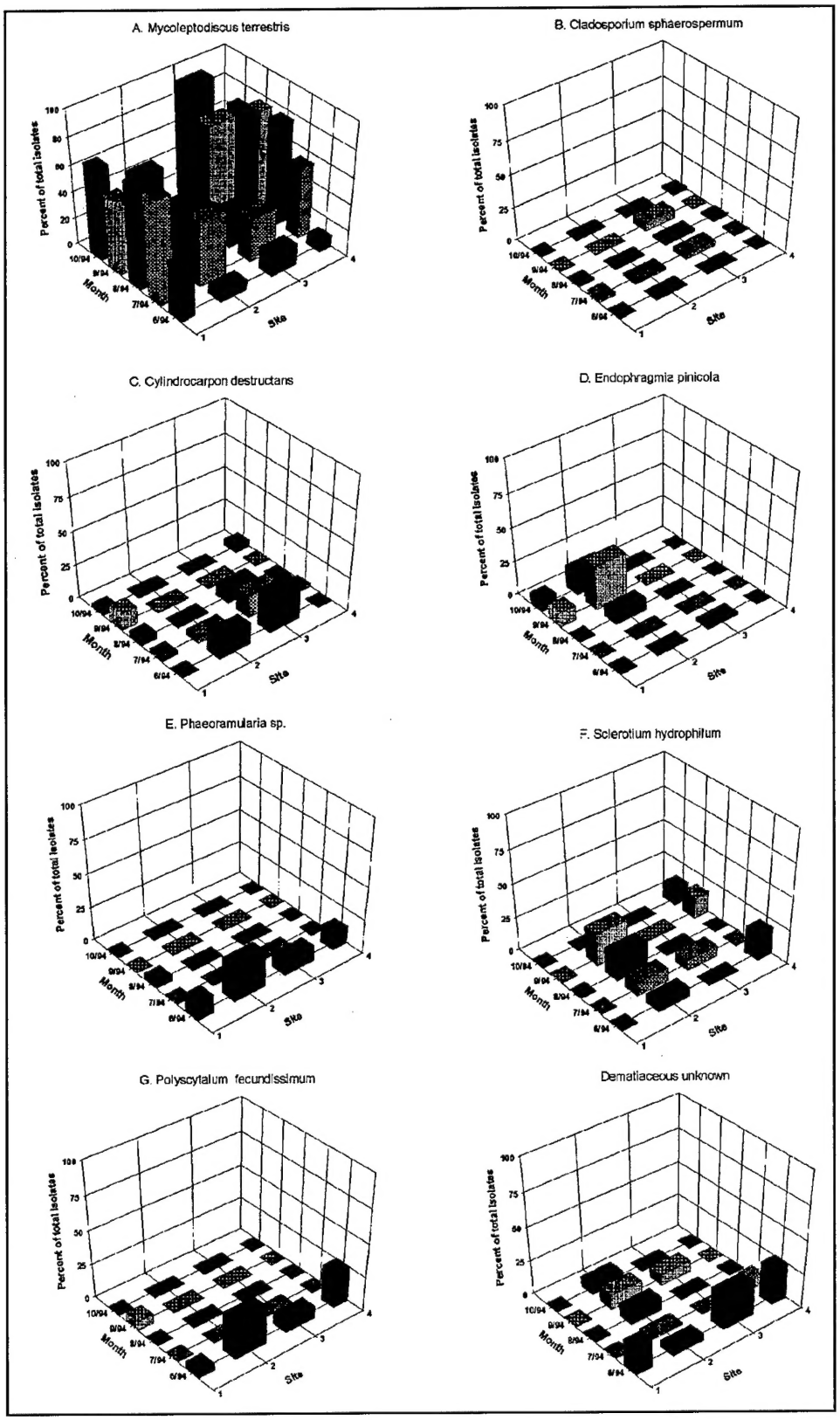

Figure 2. Relative frequency of eight fungal species isolated from milfoil shoot tissue collected at four sites on Guntersville Reservoir, June-October 1994

infrequent occurrence were *Tetracladium setigerum*, *Fusarium oxysporum*, *Drechslera australiensis*, *Ramichloridium* sp., *Pythium* sp., *Epicoccum purpurascens*, and *Flagellospora curvula*.

Many of the isolates recovered from milfoil stem and leaf tissues are in genera that have been previously reported to be endophytic. *Acremonium*, *Glomerella*, *Phomopsis*, *Cladosporium*, *Penicillium*, *Nigrospora*, *Colletotrichum*, *Fusarium*, and *Pestalotiopsis* recorded in the present study are genera that have a number of endophytic species (Andrews, Hecht, and Bashirian 1982; Bills and Polishook 1992b; Petrini 1991; Rodriguez and Redman 1997). In addition, the teleomorphs *Emericellopsis* and *Plectosphaerella* have anamorphic states *Acremonium* and *Fusarium*, respectively, that are endophytic. Several species isolated from milfoil during this study have been reported from leaves and stems of healthy and senescent woody and herbaceous vegetation. These include *Curvularia pallescens*, *Polyscytalum fecundissimum*, *Endophragmia pinicola*, *Phaeoramularia* sp., *Leptosphaeria coniothyrium*, *Papulaspora immersa*, *Cylindrocarpon destructans*, *Zygosporium masonii*, and *Microsphaeropsis olivacea*. Other species found on milfoil that could potentially be pathogens or latent pathogens include *Sclerotium hydrophilum*, *Phoma* sp. and *Pythium* sp.

The pattern of colonization of *M. terrestris* in milfoil is similar to that of *M. atromaculans* in Atlantic white cedar. Bills and Polishook (1992b) found *M. atromaculans* to be the second most frequently isolated endophyte from asymptomatic leaves of Atlantic white cedar. Frequent reisolations of the fungus from trees at one site in the following year indicated that the population was persistent through time (Bills and Polishook 1992a). *Mycoleptodiscus terrestris* was isolated from milfoil tissues at high frequencies in successive years on Guntersville Reservoir. It has also been the dominant species collected on several occasions from the same sites in Vermont, Minnesota, Illinois, Tennessee, Kentucky, and Alabama (unpublished).

The extent of milfoil tissue colonization increased from June to October both in sites 1 and 2, where plants were stressed from conditions of high turbidity and low light during 1994 and in sites 3 and 4, where plants remained green and asymptomatic until tissues began to senesce in September. This pattern seems to be consistent with endophytes that are often referred to as opportunists or latent pathogens that manifest themselves only when their host plants are weakened through stress or senescence (Dorworth and Callan 1996; Sinclair and Cerkauskas 1996).

Many of the isolates represent new reports of fungi from milfoil in the United States. A *Pythium* sp. from Florida is the only report from this host in the index compiled by Farr et al. (1989). Other reports include *Physoderma* sp. (Sparrow 1974) *Fusarium sporotrichioides* (Andrews and Hecht 1980), *Acremonium curvulum* (Andrews, Hecht, and Bashirian 1982), and *M. terrestris* (Gunner 1983). Bernhardt (1983) identified 12 species of fungi isolated from milfoil collected in California. In common with species collected in the present study were *Phoma* sp., *Acremonium* sp., and *Flagellospora curvula*. The most extensive reports of fungi that occur on milfoil have come from studies in Europe (Lekic 1971; Harvey and Evans 1997) and in China (Shearer 1997). It is interesting to note that *M. terrestris* was absent from species lists compiled from Europe but was isolated frequently on asymptomatic and stressed milfoil in China.

The consistent and repetitive isolation of *M. terrestris* from asymptomatic and stressed milfoil populations provides good evidence that the fungus occurs as an endophyte within host tissues. It is not currently known if the presence of the fungus imparts to the plant added resistance to herbivory or ingress from pathogenic organisms. The high frequency of isolation of *M. terrestris* from milfoil populations that are stressed or undergoing senescence suggests it is a class-three endophyte (Rodriguez and Redman 1997) or latent pathogen. Additional studies that examine senescence and stress factors such as herbivory, chemical runoff, and turbidity on milfoil populations will help to elucidate how endophytic fungi such as *M. terrestris* affect the growth and survival of milfoil.

Literature Cited:

Agrios, G. N. (1988). *Plant Pathology*. 3rd ed. Academic Press, New York.

Andrews, J. H., and Hecht, E. P. (1980). "Evidence for pathogenicity of *Fusarium sporotrichioides* to Eurasian water milfoil, *Myriophyllum spicatum*." *Can. J. Bot.* 59, 1069-1077.

Andrews, J. H., Hecht, E. P., and Bashirian, S. (1982). "Association between the fungus *Acremonium curvulum* and Eurasian water milfoil, *Myriophyllum spicatum*," *Can. J. Bot.* 60, 1216-1221.

Belesky, D. P., and Malinowski, D. P. (2000). "Abiotic stresses and morphological plasticity and chemical adaptations of *Neotyphodium*-infected tall fescue plants." *Microbial Endophytes.* C. W. Bacon and J. F. White Jr., ed., Marcel Dekker, New York, 455-484.

Bernhardt, E. A. (1983). "A survey for pathogens of aquatic weeds in California for use in biological control," Ph.D. diss., Department of Plant Pathology, University of California, Davis.

Bills, G. F., and Polishook, J. D. (1992a). "A new species of *Mycoleptodiscus* from living foliage of *Chamaecyparis thyoides*," *Mycotaxon.* 43, 453-460.

Bills, G. F., and Polishook, J. D. (1992b). "Recovery of endophytic fungi from Chamaecyparis thyoides," *Sydowia.* 44, 1-12.

Booth, C. (1966). *The genus Cylindrocarpon.* Mycological Papers, No. 104. Commonwealth Mycological Institute, Kew, Surrey, U.K.

Booth, C. (1971). *The genus Fusarium.* The Eastern Press Limited, London.

Carmichael, J. W., Kendrick, W. Bryce, Conners, I. L., and Sigler, L. (1980). *Genera of Hyphomycetes.* University of Alberta Press, Edmonton, Alberta, Canada.

Carroll, G. C. (1986). "The biology of endophytism in plants with particular reference to woody perennials." *Microbiology of the phylloplane.* N. J. Fokkema and J. Van den Heuvel ed., Cambridge University Press, Cambridge, 205-222.

Carroll, G. C. (1991). "Beyond pest deterrence—Alternative strategies and hidden costs of endophytic mutualisms in vascular plants." *Microbial Ecology of Leaves.* J. H. Andrews and S. S. Hirano, ed., Springer-Verlag, NY, 358-375.

Clay, K. (1988). "Clavicipitaceous fungal endophytes of grasses: coevolution and the change from parasitism to mutualism." *Co-evolution of fungi with plants and animals.* D. L. Hawksworth and K. Pirozynski, ed., Academic Press, London, 79-105.

Clay, K. (1991). "Fungal endophytes, grasses, and herbivores." *Microbial mediation of plant-herbivore interactions.* B. Barbosa, V. A. Krischik, and C. G. Jones, ed., 199-252.

Clay, K. (1997). "Fungal endophytes, herbivore and the structure of grassland communities." *Multitrophic interactions in terrestrial systems*. A. C. Gange and V. K. Brown, ed., Blackwell Science 151-169.

De Bary, A. (1866). "Morphologie und physiologie der pilze. Flechten und Myxomyceten." Vol II. *Hofmeister's handbook of physiological botany*. Leipzig.

Dhingra, O. E., and Sinclair, J. F. (1995). *Basic plant pathology methods*. CRC Press, Inc., Boca Raton, FL.

Domsch, K. H., Gams, W., and Anderson, T. (1980). *Compendium of soil fungi*. Vol I. Academic Press, London.

Dorworth, C. E., and Callan, B. E. (1996). "Manipulation of endophytic fungi to promote their utility as vegetation biocontrol agents." *Endophytic fungi in grasses and woody plants*. S. C. Redlin and L. M. Carris, ed., APS Press, St Paul, MN, 209-216.

Ellis, M. B. (1971). *Dematiaceous Hyphomycetes*. Cambrian Printers, Aberystwyth, Dyfed, U.K.

Ellis, M. B. (1976). *More Dematiaceous Hyphomycetes*. Cambrian Printers, Aberystwyth, Dyfed, U.K.

Faeth, S. H., and Hammon, K. E. (1997). "Fungal endophytes in oak trees: Long-term patterns of abundance and associations with leafminers." *Ecology*. 78, 810-819.

Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. (1989). *Fungi on plant and plant products in the United States*. American Phytopathological Society, St. Paul, MN.

Gams, W. (1971). *Cephalosporium-srtige Schimmelpilze (Hyphomycetes)*. Gustav Fischer Verlag, Stuttgart.

Gunner, B. H. (1983). "Microbial control of Eurasian watermilfoil," Miscellaneous Paper A-83-4, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Harvey, J. L., and Evans, H. C. (1997). "Assessment of fungal pathogens as biocontrol agents of *Myriophyllum spicatum*," Miscellaneous Paper A-97-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Lane, G. A., Christensen, M. J., and Miles, C. O. (2000). "Coevolution of fungal endophytes with grasses: The significance of secondary metabolites." *Microbial endophytes*. C. W. Bacon and J. F. White, Jr., ed., Marcel Dekker, New York, 341-375.

Lekic, M. (1971). *Ann. Rep. Inst. Plant. Prot.*, Beograd.

Petrini, O. (1991). "Fungal endophytes of tree leaves." *Microbial ecology of leaves*. J. H. Andrews and S. S. Hirano, ed., Springer-Verlag, New York, 179-187.

Pitt, John I. (1979). *The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces*. Academic Press, London.

Rodriguez, R. J., and Redman, R. S. (1997). "Fungal life-styles and ecosystem dynamics: Biological aspects of plant pathogens, plant endophytes and saprophytes." *Advances in botanical research*. 24, 170-193.

Shearer, J. F. (1997). "Classical pathogen biocontrol research in Asia 1994-1995: Surveys for pathogen agents of *Hydrilla verticillata* (L. f.) Royle and *Myriophyllum spicatum* L.," Technical Report A-97-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Sinclair, J. B., and Cerkauskas, R. F. (1996). *Endophytic fungi in grasses and woody plants*. S. C. Redlin and L. M. Carris, eds., APS Press, St. Paul, MN, 3-29.

Sparrow, F. K. (1974). "Observations on chytridyaceous parasites of Phanerograms. XIX. A *Physoderma* on Eurasian water Milfoil (*Myriophyllum spicatum*)." *Amer. J. Bot.* 61, 174-180.

Sridhar, K. R., and Raviraja, N. S. (1995). "Endophytes-A crucial issue," *Current Science* 69, 570-571.

Stone, J. K., Bacon, C. W., and White, J. F., Jr. (2000). "An overview of endophytic microbes: Endophytism defined," *Microbial endophytes.* C. W. Bacon and J. F. White, Jr., ed., Marcel Dekker, New York, 3-29.

Sutton, B. C. (1980). *The Coelomycetes.* Commonwealth Mycological Institute, Kew.

AD NUMBER	DATE	DTIC ACCESSION
1. REPORT IDENTIFYING INFORMATION		REQ
A. ORIGINATING AGENCY US Army Engineer Res & Dev Center		1. Put on r 2. Con
B. REPORT TITLE AND/OR NUMBER ERDC TN-APCRP-BC-03		3. Atta me 4. Use int
C. MONITOR REPORT NUMBER		5. Do fo
D. PREPARED UNDER CONTRACT NUMBER		DTI
2. DISTRIBUTION STATEMENT Approved for public release; distribution is unlimited.		1. A 2. R

20010515 043

DTIC Form 50
JUL 96

PREVIOUS EDITIONS ARE OBSOLETE